



<110> Leeflang, Chris  
Van der Kleij, Wilhelmus A.H.

<120> Methods for Site-Directed Mutagenesis  
and Targeted Randomization

<130> GC787-2-US

<140> US 10/541,737

<141> 2005-07-08

<150> PCT/US04/01334

<151> 2004-01-16

<150> US 60/440,792

<151> 2003-01-16

<160> 7

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 275

<212> PRT

<213> Bacillus amyloliquefaciens

<400> 1

Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu  
1 5 10 15

His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp  
20 25 30

Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala  
35 40 45

Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His  
50 55 60

Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly  
65 70 75 80

Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu  
85 90 95

Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu  
100 105 110

Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly  
115 120 125

Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala  
130 135 140

Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly  
145 150 155 160

Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala  
165 170 175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val  
180 185 190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr  
195 200 205

Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser  
210 215 220

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn  
225 230 235 240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys  
245 250 255  
Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
260 265 270  
Ala Ala Gln  
275

<210> 2  
<211> 275  
<212> PRT  
<213> *Bacillus subtilis*

<400> 2  
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His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp  
20 25 30  
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala  
35 40 45  
Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His  
50 55 60  
Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly  
65 70 75 80  
Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu  
85 90 95  
Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu  
100 105 110  
Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly  
115 120 125  
Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser  
130 135 140  
Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly  
145 150 155 160  
Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala  
165 170 175  
Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala  
180 185 190  
Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr  
195 200 205  
Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr  
210 215 220  
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr  
225 230 235 240  
Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr  
245 250 255  
Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
260 265 270  
Ala Ala Gln  
275

<210> 3  
<211> 274  
<212> PRT  
<213> *Bacillus licheniformis*

<400> 3  
Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val

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Gln	Ala	Gln	Gly	Phe	Lys	Gly	Ala	Asn	Val	Lys	Val	Ala	Val	Leu	Asp
				20		25								30	
Thr	Gly	Ile	Gln	Ala	Ser	His	Pro	Asp	Leu	Asn	Val	Val	Gly	Gly	Ala
				35		40							45		
Ser	Phe	Val	Ala	Gly	Glu	Ala	Tyr	Asn	Thr	Asp	Gly	Asn	Gly	His	Gly
				50		55					60				
Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asp	Asn	Thr	Thr	Gly	Val
	65				70				75			80			
Leu	Gly	Val	Ala	Pro	Ser	Val	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu	Asn
				85				90			95				
Ser	Ser	Gly	Ser	Gly	Ser	Tyr	Ser	Gly	Ile	Val	Ser	Gly	Ile	Glu	Trp
				100				105			110				
Ala	Thr	Thr	Asn	Gly	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Ala
				115			120				125				
Ser	Gly	Ser	Thr	Ala	Met	Lys	Gln	Ala	Val	Asp	Asn	Ala	Tyr	Ala	Arg
				130			135				140				
Gly	Val	Val	Val	Val	Ala	Ala	Gly	Asn	Ser	Gly	Asn	Ser	Gly	Ser	
	145				150			155			160				
Thr	Asn	Thr	Ile	Gly	Tyr	Pro	Ala	Lys	Tyr	Asp	Ser	Val	Ile	Ala	Val
				165			170			175					
Gly	Ala	Val	Asp	Ser	Asn	Ser	Asn	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly
				180			185			190					
Ala	Glu	Leu	Glu	Val	Met	Ala	Pro	Gly	Ala	Gly	Val	Tyr	Ser	Thr	Tyr
				195			200			205					
Pro	Thr	Asn	Thr	Tyr	Ala	Thr	Leu	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro
				210			215			220					
His	Val	Ala	Gly	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Leu	
	225				230			235			240				
Ser	Ala	Ser	Gln	Val	Arg	Asn	Arg	Leu	Ser	Ser	Thr	Ala	Thr	Tyr	Leu
				245			250			255					
Gly	Ser	Ser	Phe	Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn	Val	Glu	Ala	Ala
				260			265			270					

Ala Gln

<210> 4  
<211> 269  
<212> PRT  
<213> *Bacillus latus*

<400> 4															
Ala	Gln	Ser	Val	Pro	Trp	Gly	Ile	Ser	Arg	Val	Gln	Ala	Pro	Ala	Ala
1				5			10						15		
His	Asn	Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp
				20			25			30					
Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly	Gly	Ala	Ser
				35			40			45					
Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr
				50			55			60					
His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu
				65			70			75			80		
Gly	Val	Ala	Pro	Ser	Ala	Glu	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala
				85			90			95					
Ser	Gly	Ser	Gly	Ser	Val	Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala
				100			105			110					
Gly	Asn	Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser	Pro	Ser
				115			120			125					

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly  
130 135 140  
Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser  
145 150 155 160  
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln  
165 170 175  
Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile  
180 185 190  
Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr  
195 200 205  
Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala  
210 215 220  
Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile  
225 230 235 240  
Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu  
245 250 255  
Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg  
260 265

<210> 5  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer

<400> 5 27  
gtgtgtggc ccatcagtct gacgacc

<210> 6  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer

<400> 6 27  
gtgtgtggc cctattcgga tatttag

<210> 7  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer

<400> 7 27  
gtgtgtggc ccatcagtct cacgacc